Journal of Medicinal Plants Research Vol. 6(14), pp. 2777-2788, 16 April, 2012
Available online at http://www.academicjournals.org/JMPR
DOI: 10.5897/JMPR11.11-1025
ISSN 1996-0875 ©2012 Academic Journals

Full Length Research Paper

Genetic evaluation of *Andrographis paniculata* (Burm. f.) Nees revealed by SSR, AFLP and RAPD markers

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Accepted 26 August, 2011

***Andrographis paniculata* (Burm. f.) Nees is a medicinal plant widely used in Thailand and many countries for therapeutic value. The present study was aimed to investigate its genotypic variations in various locations of Thailand based on simple sequence repeats (SSR), amplified fragment length polymorphism (AFLP) and random amplified polymorphic dna (RAPD) marker system. SSR, AFLP and RAPD were compared in terms of their informativeness and efficiency in a study of genentic diversity and relationships among 58 samples of *A. paniculata* in Thailand. All three techniques presented highly correlated results, with a correlation coefficient of r = 0.996. The low values of expected heterozygosity were obtained for all marker systems due to their capacity to reveal the low number of polymorphic bands in each markers system. All marker systems also gave very high genetic similarity values, which represent the values that are higher than 0.9; the highest is SSR (0.989) followed by AFLP (0.964) and RAPD (0.905), respectively. The cophenetic correlation coefficients of similarity were statistically significant (r = 0.996, p < 0.001) thus implying the correspondence for all combination of three marker systems. The results obtained indicated that the distribution of *A. paniculata* among each region of Thailand are likely to belong to the same variety and are relatively undifferentiated across a large geographic rang.**

Key words: *Andrographis paniculata*, SSR, AFLP, RAPD, genetic similarities.

INTRODUCTION

Thailand belongs to one of the twelve megadiversity centers which has immense biotic wealth marked by remarkable ecosystem, species and genetic diversity. This rich genetic diversity is matched equally by rich cultural diversity including the use of various Thai medicinal plants in health care traditions or Thai traditional medicine (TTM).

The species variations of Thai medicinal plant may be plastic in nature, often induced by the environment or sometime may have genetic basis. For efficient conservation and management of medicinal plant diversity, the genetic composition of species collected from different phytogeographical regions needs to be measured. Identification and determination of the genetic variation within populations of selected medicinal plant in Thailand and Asia.

A. paniculata (Burm. f.) Nees (Acanthaceae*)*, commonly known as Fha-Tha-Laai-Joan, has been widely used in Thailand and other countries for the treatment of the common cold, fever and non-infectious

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diarrhoea (Thanagkul and Chaichantipayut, 1985; Vedavathy and Rao, 1991; Caceres et al., 1999). In addition, *A. paniculata* has been frequently used for treatment of upper respiratory tract infections, fever, sore throat, inflammation and diarrhea (Techadamrongsin et al., 1999). Past research found various pharmacological activities of *A. paniculata*, including immune-stimulatory (Puri et al., 1993), anti-HIV (Otake et al., 1995; Calabrese et al., 2000), hepatoprotective (Handa and sharm, 1990; Kapil et al., 1993) anti-malarial (Misra et al., 1992) and cardiovascuta (Zhang and Tan, 1997; Zhang et al., 1998).

SSR (microsatellite) occur frequently in most eukaryote genomes and can be very informative, multi allelic and reproducible (Vos et al., 1995). The application of SSR technique, however, is limited to availability of suitable microsatellite markers, which have been developed for species such as rice (Zhao and Kochert, 1993), soybean (Rongwen et al., 1995) and maize (Taramino and Tingey, 1996). In *A. paniculata*, no previous report has yet been applied. AFLP is the generation of multiple marker bands in a single assay without prior knowledge of deoxyribonucleic acid (DNA) sequences. In *A. paniculata*, the AFLP technique also has not yet been applied. RAPD have been utilized for numerous purposes in plants, with RAPD due to the ease of detection and speed of use. (Tingey and del Tufo 1993). Comparisons of molecular marker systems for measuring genetic diversity have been reported in several plant species (Powell et al., 1996; Milbourne et al., 1997; Russell et al., 1997; Pejic et al., 1998; Crouch et al., 2000; Garcia-Mas et al., 2000; Staub et al., 2000) but no such studies have yet been reported in *A. paniculata.*

The objectives of the present study are to find out genotypic variations of *A. paniculata* growing in various locations of Thailand and to quantify its patterns of population differentiation.

In this study, possible genetic variation among *A. paniculata* collected from different geographical locations was analyzed using molecular markers, SSR, AFLP and RAPD to examine genotypic diversity of *A. paniculata* collections in Thailand.

MATRIALS AND METHODS

Plant material

Fifty-eight accessions of *A. paniculata* were collected from 5 populations of geographic locations in Thailand, consisting of 8 accessions from North, 24 from Northeast, 4 from East, 11 from central and 11 from South of Thailand (Table 1).

DNA extraction

Total genomic DNA of *A. paniculata* was isolated from fresh young leaves using the cetyltrimethylammonium bromide (CTAB) modified protocol (Murray and Thompson, 1980) at the DNA Technology Laboratory, Kasetsart University, Kamphaengsaen Campus, Thailand.

Marker analytical technique

SSR technique

Seventy SSR markers (SSR primer pairs: Ap01 to Ap70) used in this study were previously developed by our Laboratory (unpublished) for genetic studies in *A. paniculata.* For preliminary testing of SSR, total genomic DNA from each individual was pooled for six groups. Amplification and detection were performed using polymerase chain reaction.

The thermo-cycling conditions are as followings: an initial denaturation at 94°C for 30 min was followed by 44 cycles of 30 s at 94°C, 30 s at 55°C, plus 30 s at 62°C for Ap68, Ap69 and Ap70 and 1 min at 72°C with a final extension for 5 min at 72°C. Polymerase chain reaction (PCR) was conducted in 10 µl volume containing 5 ng of template DNA, 5 µM of primer (each), 1 mM dNTPs (each), 10x *Taq* buffer and 1 units *Taq* polymerase (Promega®). Amplification fragments were separated by electrophoresis through a 6% denaturing polyacrylaminde gel and visualized by silver-staining as described by Bassam et al. (1991). Alleles were scored according to molecular weight and between-gel differences were controlled by the inclusion of molecular weight markers on each gel.

AFLP technique

AFLP markers were developed following the method of Vos et al. (1995). The 200 mg of genomic DNA was digested by restriction enzymes *Eco*RI and *Mse*I. Two polymerase chain reaction linker sequences were ligated onto the resulting pool of DNA fingerprintings. The ligation mixture was diluted 1: 20, and 5 ul of this dilution was used for PCR preamplification in a 10 µl volume with 50 ng of each preamplification primer. The preamplification products were also diluted 1:20, and 5 µl of this dilution was used as the template for selective amplification in 10 µl reactions with 25 ng each of two selective primers. Four primer combinations obtained by the primer pairs E-AAG/M-ACT, E-ACC/M-CTG, E-GTA/M-CAA AND E-CGT/M-TAC was adequate to discriminate all genotypes.

PCR analyses were performed in a PTC-200 Peltier Thermal Cycler (9600 Thermal Cycler ; Perkin Elmer) programmed to perform with one cycle of 94°C, 60 s at annealing temperature of 56°C, and 60 s of DNA extension at 72°C and 5 min final extension. The fragment size of the PCR products was checked by 4.5% denaturing Polyacrylamide Gel Electrophoresis, stained with silver staining and the ∅x *Hin*fI was loaded parallelly to the products as standard marker.

RAPD technique

The RAPD amplifications were carried out using thirty random primers (Operon Technologies, Alameda, CA, USA). Eight primers (primer pair: OPZ-01, 04, 06, 08, 10, 12, 16 and OPW-05) showing reproducible polymorphism were selected. RAPD reactions were done in a total volume of 15 µl containing 200 µM of dNTPs, 2.5 mM $MgCl_2$, 0.3 U *Taq* DNA polymerase, 5 pmol of each primer and 50 ng of DNA Template. DNA amplification was performed in a DNA Thermal Cycler 600 (Applied Biosystems) in 0.5 ml PCR tubes programmed for initial denaturation at 93°C for 1 min, 1 min at 35°C and 2 min at 72°C, followed by 44 cycles for 1 min at 95°C, at annealing temperature of 35°C for 1 min and at 72°C for 2 min as an extension step. The final extension step was done for 10 min at 72°C and the reactions were kept at soak file at 4°C. Amplification products were subsequently separated on 15% agarose gel with

Table 1. Geographical localities and abbreviations of the *A. paniculata* population used.

Accession	Origin of geographical distribution	Region	Code
1	Kanchanaburi	Central	KN
2	Nakorn pathom	Central	KPS
3	Nakorn pathom	Central	NP
4	Chachoengsao	Central	CC
5	Chumphon	Southern	CP
6	Prachuab Khiri Khan	Central	PC1
7	Phetchaburi	Central	PB
8	Prachuab Khiri Khan	Central	PC2
9	Nakorn Ratchasima	Northeastern	NR1
10	Prachin Buri	Central	PR1
11	Prachin Buri	Central	PR2
12	Trat	East	TD
13	Chantaburi	East	CT
14	Rayong	East	RA1
15	Rayong	East	RA2
16	Surin	Northeast	SR
17	Buriram	Northeast	BR1
18	Buriram	Northeast	BR2
19	Amnat Charoen	Northeast	AC
20	Ubon Ratchathani	Northeast	UB1
21	Ubon Ratchathani	Northeast	UB2
22	Khon Kaen	Northeast	KK1
23	Maha Sarakham	Northeast	MK
24	Si Saket	Northeast	SK1
25	Si Saket	Northeast	SK2
26	Roi Et	Northeast	RE
27	Satun	South	ST
28	Songkla	South	SO1
29	Songkla	South	SO2
30	Phattalung	South	PL
31	Trang	South	Tr
32	Krabi	South	KB1
33	Phang Nga	South	PN
34	Krabi	South	KB2
35	Nakhon Si Thammarat Krabi	South	NS
36	Suratthani	South	SRT
37	Chaiyaphum	Northeast	CY1
38	Chaiyaphum	Northeast	CY2
39	Loei	Northeast	LO1
40	Loei	Northeast	LO2
41	Udon Thani	Northeast	UD1
42	Nong Bua Lam phu	Northeast	NB
43	Chiang Mai	North	CM1
44	Chiang Mai	North	CM2
45	Chiang Rai	North	CR1
46	Chiang Rai	North	CR2
47	Sing Buri	Central	SB
48	Utlaradit	North	UT
49	Lamphang	North	LP
50	Nong Khai	Northeast	NK1
51	Phayao	North	PY

Table 1. Contd.

52	Sukhothai	North	ST1
53	Udon Thani	Northeast	UD2
54	Saraburi	Central	SA
55	Kalasin	Northeast	KS
56	Nong Khai	Northeast	NK2
57	Nakorn Ratchasima	Northeast	KK2
58	Khon Kaen	Northeast	KK2

Table 2. Level of polymorphism and comparison of informativeness obtained with SSR, AFLP and RAPD markers in 58 samples of *A. paniculata.*

	Marker system		
Parameters	**SSR**	**AFLP**	**RAPD**
Number of assay units	70	4	8
Average number of bands perassay unit	1.31	21.5	8
Number of polymorphic bands	6	15	46
Number of loci	92	85	56
Expected heterozygosity (H_e)	0.067	0.050	0.21
Effective number of alleles per locus (n_e)	0.011	0.019	1.018
Assay efficiency index (A_i)	0.000157	0.0048	0.145

ethidium bromide staining.

Data analysis

Each gel was analyzed by manually scoring the presence (1) or absence (0) of bands in individual lanes. Binary data accessions were evaluated by estimating the Jaccard's coefficient (Legendre and Legendre, 1983), and the accessions were clustered by the unweighted pair-group method with arithmetic averages (UPGMA). The dendrograms generated on the basis of the above indexes were compared by computing the co-phenetic correlation (Sneath and Sokal, 1973). A cophenatic value matrix of the UPGMA clustering was also used to test for the goodness of fit of the clustering to the similarity matrix on which it was based by computing the product- moment correlation *r* (Mentel, 1967). Genetic similarity among all accessions was calculated using the Jaccard's coefficient (Jaccard, 1908) within NTSYS-pc software (Rohlf, 1993).

RESULTS

Level of Polymorphism

The 58 samples of *A. paniculata* were investigated using the SSR, AFLP and RAPD marker systems as described. All of the molecular markers were provided a similar fingerprinting pattern each for the individual cultivars among the geographical distributions.

Fewer than three marker system analyses, 13,507 bands were observed across all collecting samples. However, only 46 bands were polymorphic. The total number of polymorphic bands rang from 6 for SSR, 15 for AFLP and 25 for RAPD. Surprisingly, the total number of loci scored for entirely three marker systems was relatively high. The percentage of polymorphic bands obtained for each assay unit was relatively low, with 0.001, 0.003 and 0.077% of them being polymorphic. The levels of polymorphism detected with each marker system are summarized in Table 2.

Comparison of informativeness obtained with SSRs, AFLPs and RAPDs markers.

Information content, measured as expected heterozygosity (H_e) was highest for the AFLP marker, followed by the RAPD method and finally SSR. For SSR system, only two out of seventy primer pairs, Ap01 and Ap02, revealing 2.8% of the total number of SSR primers used, generated polymorphic bands. Very low expected heterozygosity was observed for all of three marker systems. An average number of 0.07 allels per locus, with an effective number of allele per locus (n_e), could be detected for SSR at 0.011. These values did correlate with the value obtained from AFLP and RAPD at 0.012 and 0.018, respectively. This was reflected also in very lower expected heterozygosity. The very low assay efficiency index (A_i) values were observed for all three marker systems. The highest values of assay efficiency index for RAPD marker systems highlight the distinctive nature of these markers. This is due to the simultaneous detection of several polymorphic markers per single selection. The highest A_i values were observed for RAPD (0.145), the lowest for SSR (0.0001) and the values for

Table 3. comparisons of genetic similarity estimates obtained from 3 molecular marker systems, using Jaccard's coefficient.

Markers	Minimum	Maximum	Average
SSR	0.978	1.000	0.989
AFLP	0.927	1.000	0.964
RAPD	0.809	1.000	0.905

AFLP (0.004) markers were intermediate between those of RAPD and SSR. However, the values obtained from all three marker systems were still low. The informativeness obtained with each marker system is summarized in Table 2.

Genetic similarity and relationships

A summary of the genetic similarity calculated for each marker system among the 58 genomic DNA samples of *A. paniculata* collected from different location of 5 regions in Thailand is shown in Table 3. All marker systems gave very high genetic similarity values, which represent the highest value of more than 0.9 estimates for SSR, the lowest of 0.809 for RAPD and the intermediate of 0.927 for AFLP markers.

The Mentel matrix correspondence test (Mentel, 1967) was used to compare the similarity matrices. The co-phenetic correlation coefficients between the dendrogram and the original distance matrix provided for each marker system indicate the extent to statistically significant *(r<0.001)*. All the co-phenetic coefficients were extremely high, with the SSR (r = 0.97), AFLP (r = 0.99), RAPD (r = 0.94) and highest correlation in the combination data (r = 0.99).

In the UPGMA dendrogram based on the Jaccard's coefficient, all three marker systems showed an extremely high degree of genetic similarity in dendrogram topologies, though with some differences in the positioning of some collecting samples in the trees. In the SSR tree (Figure 1), only one group was observed. Among the accessions at a cut point of 90% similarity in dendrogram, some collecting samples were different in genetic similarity from other accessions such as PB, NR1, BR1 and MK. Genetic similarity values between accessions ranged from 0.98 to 0.99 with a relatively high proportion (100%) being higher than 0.95. The dendrogram obtained with AFLP markers (Figure 2) showed a similar topology as a result of SSR marker with some exceptions. In the AFLP tree, only one main group was observed but some collected sample showed a similar topology with SSR. For instance, SO1, NP, SK2, SR1, BR1, NR and MK, using 90% similarity as a cut point, show a different genetic background from other 51 accessions. However, some associations were maintained in both the the SSR and AFLP dendrograms. This was the case of BR1 and MK. For the dendrogram obtained with RAPD markers (Figure 3), at an arbitrary cut–off at 87% similarity level on the dendrogram, the plant appeared to contain two distinct cluster, A and B. Cluster A contained a large group of 55 from 58 accessions, which were all accession, except of 3 accessions that were a member group of cluster B, there are MK, RE from Northeastern region and one accession from South region of Thailand, which were SO1. However, at an arbitrary cut-off at 85% similarity level on the dendrogram, the plant revealed to only one cluster as the results of SSR and AFLP markers.

The integration analysis dendrograms among three marker types (Figure 4) constructed using the combined data of the three sets of molecular markers. The tree was still similar to those obtained separately with each marker. At an arbitrary cut-off at 95% similarity level on the dendrogram, extremely high similarity occurred in the dendrogram compared to the dendrogram derived from SSR, AFLP and RAPD markers.

DISCUSSION

Genetic similarity of *A. paniculata* collected samples

The investigation of genetic variation in *A. paniculata* in this study using three marker types found that almost all the samples collected from various locations of Thailand did not vary in genetic pattern. Although samples were collected from different regions across Thailand, few of polymorphic loci have been detected among 58 samples. Genetic similarity of each marker showed very high values in all cases, with the SSR and AFLP data resulting in the highest and the RAPD assay producing the lowest similarity value.

The 58 samples were collected from different places of varied climatic and edaphic conditions of Thailand. When brought together and sown in the same field plot under identical conditions, the 58 genotypes expressed similarity among three marker systems. These events might be due to the restricted gene flow between these populations. All the three marker systems demonstrated a clear lack of differentiation based on these groupings in Thailand. These results indicated that the distributions of *A. paniculata* among each region are likely to belong to the same variety and are relatively undifferentiated

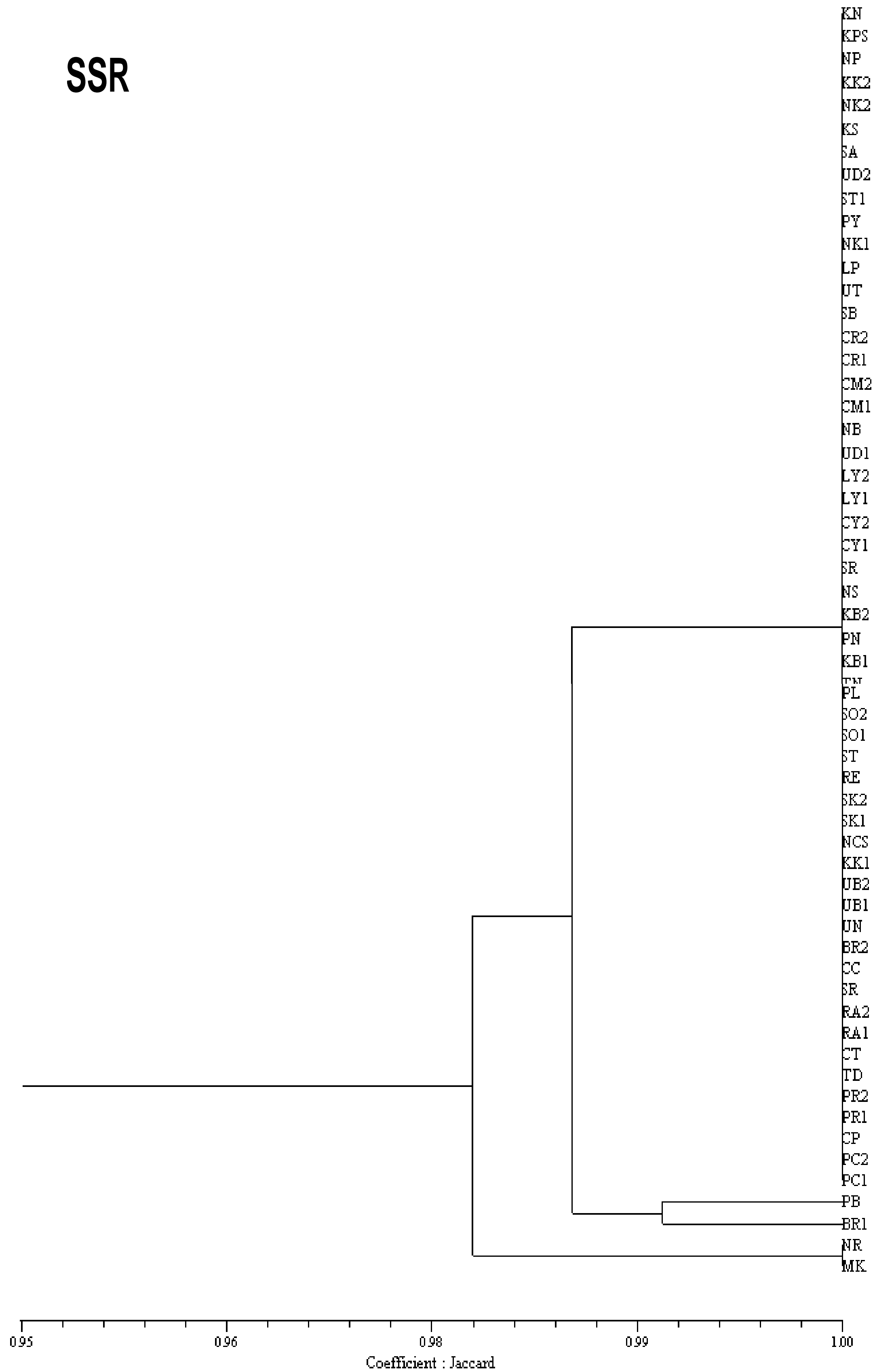

Figure 1 . Dendrograms of 58 *A. paniculata* obtained using SSR.

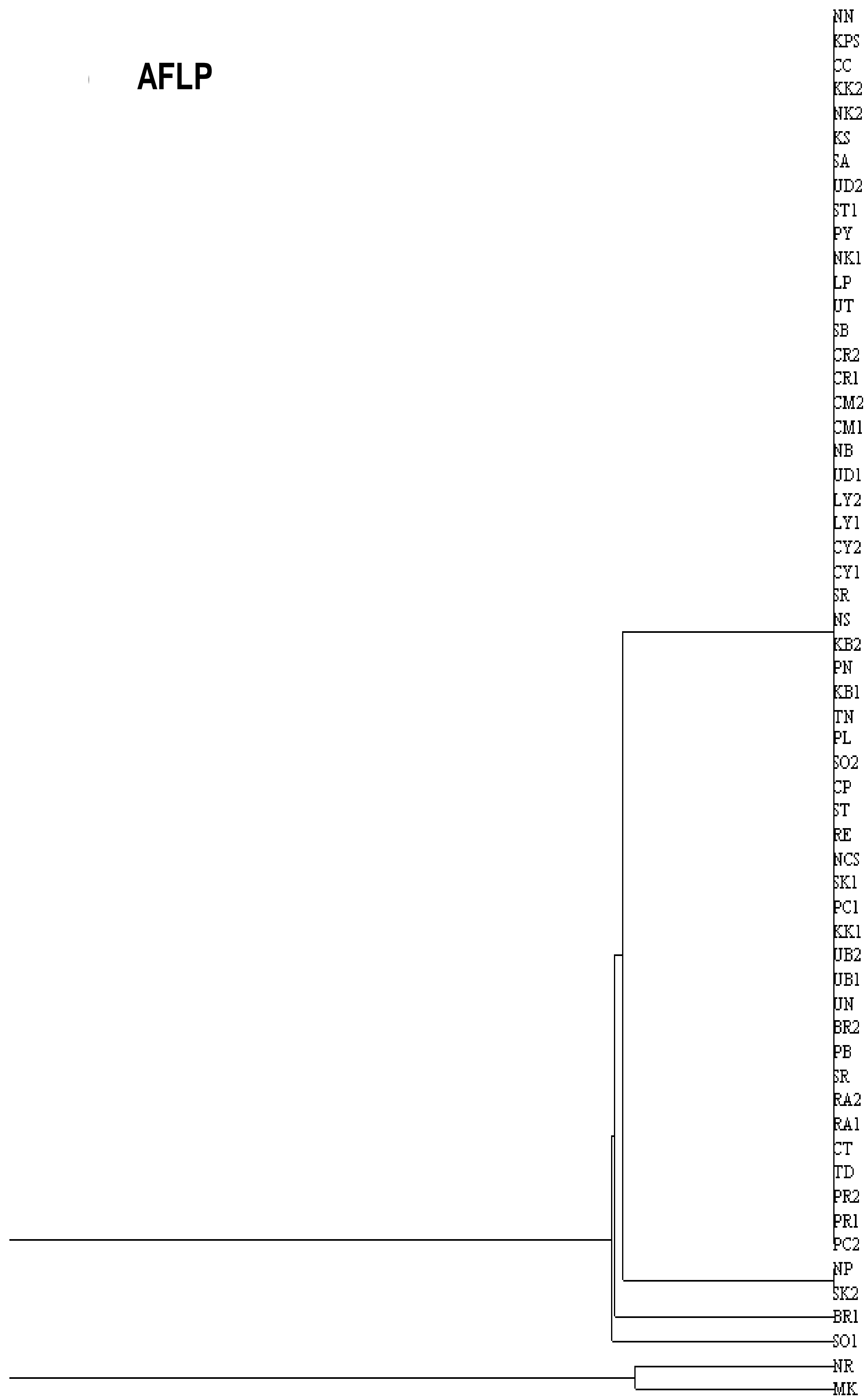

Figure 2. Dendrograms of 58 *A. paniculata* obtained using AFLP.

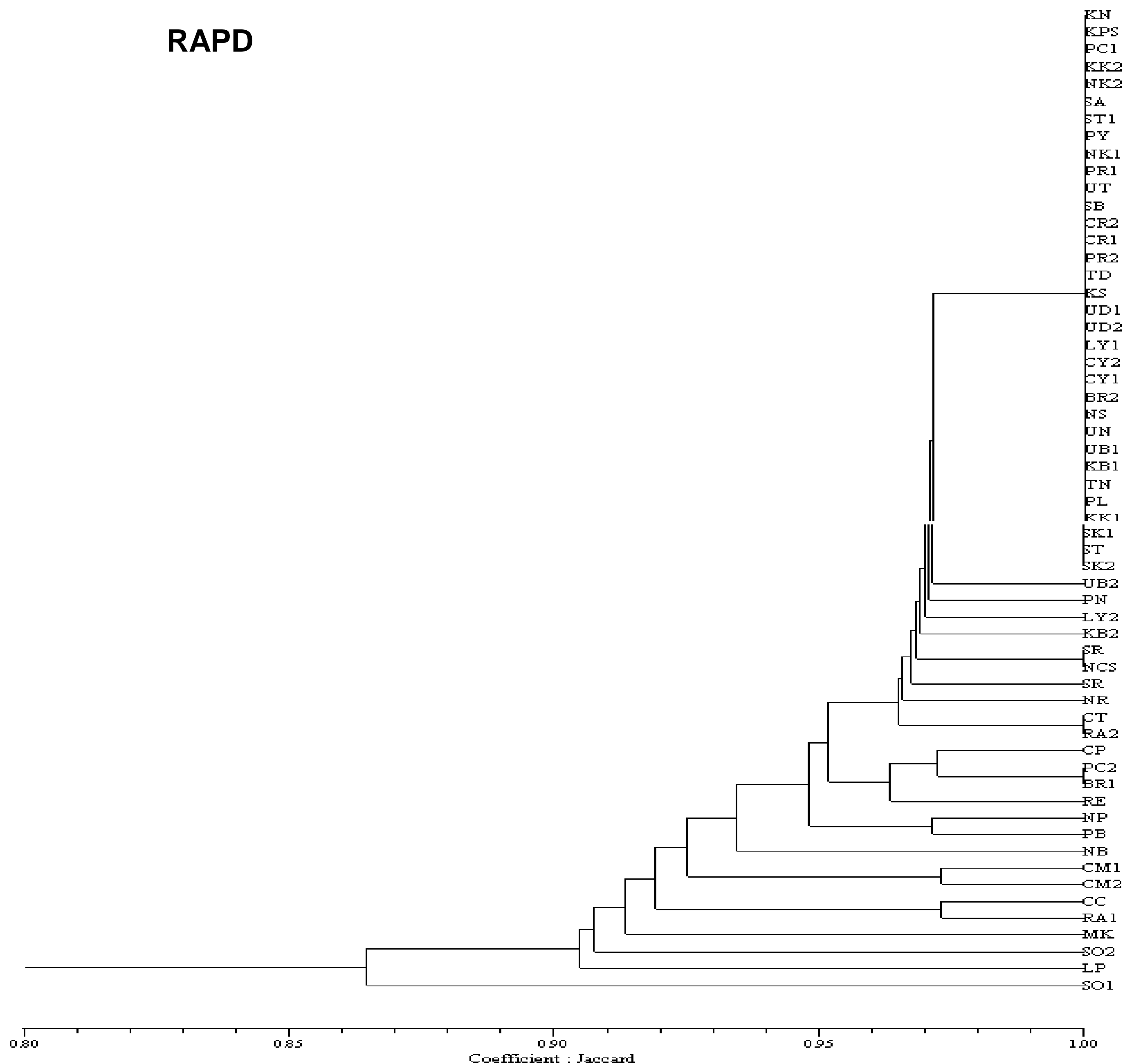

Figure 3. Dendrograms of 58 *A. paniculata* obtained using RAPD.

across a large geographic range. It is interesting to note that they were from previous reports that *A. paniculata* is probably native to India and has been introduced and cultivated as a medicinal plants in many parts of Asia (Widen et al., 1992). The result in this study corresponded to that of Padmesh et al. (1998) which reported that *A. paniculata* samples from Thailand were closely related to those from India. *A. paniculata* of Indian and Thai provenances possibly have a common origin. Having a genotype different from other accessions suggests that *A. paniculata* from Thailand is geographically distant from the rest. However, this study revealed very low level of genetic variability detected across all accessions using three marker systems. This result also corresponded to the report of Maison et al. (2005) that the genetic similarity among 25 Thai *A. paniculata* accessions using RAPD was more than 67%, and average similarity coefficient for all population was 0.83, indicating a high genetic relatedness. In addition, SSCP analysis showed little polymorphism of specific

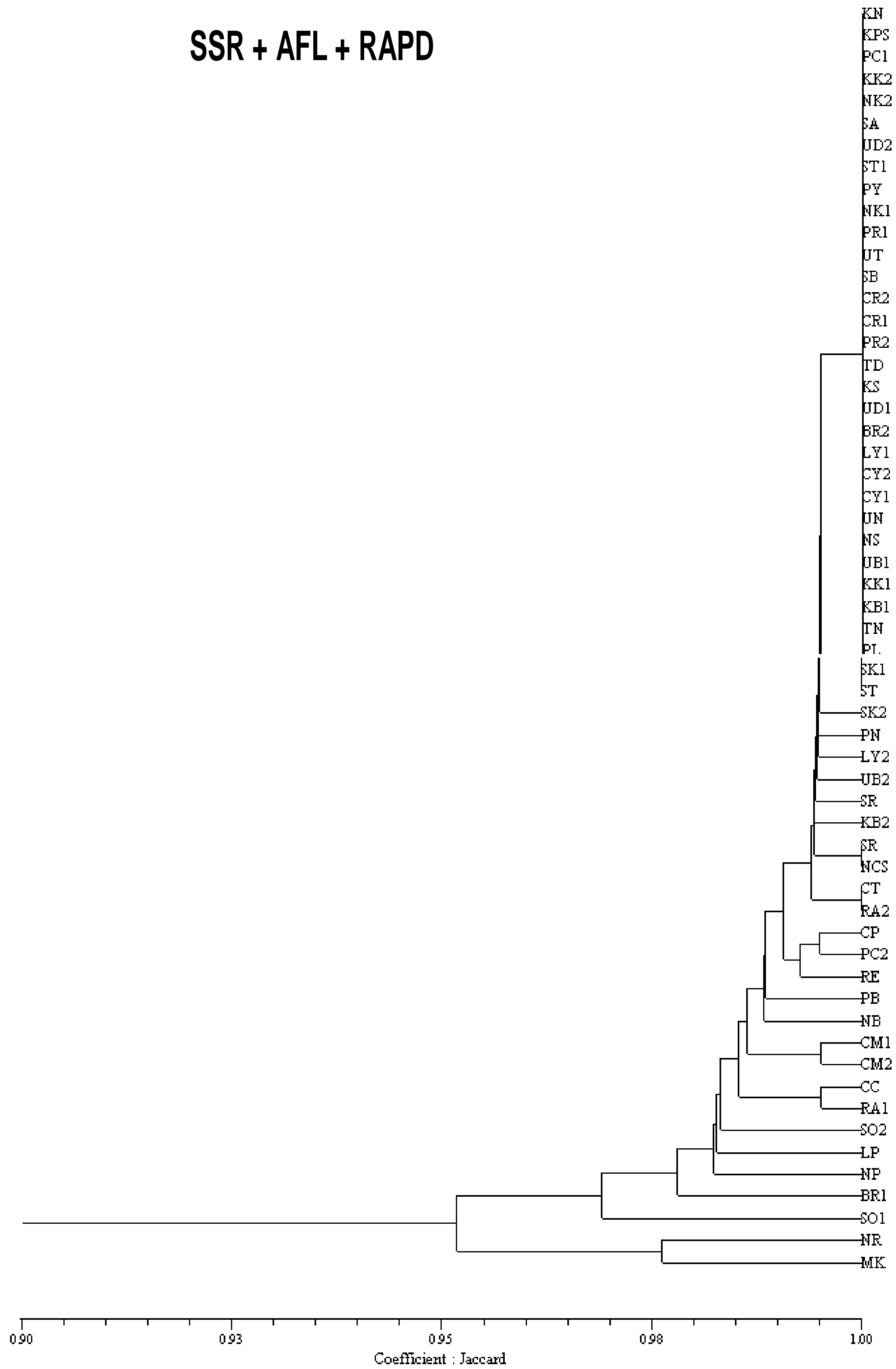

Figure 4. Dendrograms of 58 *A. paniculata* obtained using the whole data set of the three markers.

amplified products existing among these accessions. Furthermore, the similar results using molecular marker reported by Sakuanrungsirikul et al. (2008) showed no from genetic variation among *A. paniculata* accessions nine distribution areas throughout Thailand. It was reported that all loci generated by ISSR-Touchdown PCR and high annealing temperature RAPD techniques were found to be monomorphic across all accessions. The result in this study also corresponded with Lattoo et al. (2008) and Kumar (2009) which reported the high similarity values based on RAPD markers from 53 *A. paniculata* accessions in five ecogeograpic regions of India. They suggested that the extent of genetic diversity observed in each region is in conformity with breeding behavior of the species. As previously reported for genetic variability analysis including this study, all of DNA-based molecular markers techniques (RAPD, SSCP, ISSR, AFLP and SSR markers) have been found similarly proven in *A. paniculata*. It has been well explained that *A. paniculata* accessions could not detect genetic variation. It was thus possible that these species are no genetic variations which due to its being hermaphroditic, self-compatible and habitual inbreeder. Intimate proximity of adpressed stigma with the anthers and synchronization of anther dehiscence and stigma receptivity, provide for obligate autonomous selfing in the species, as found in a previous report by Latto et al. (2006). The absence of differentiation among all collecting samples in Thai *A. paniculata* may have been caused by at least three mechanisms. First, *A. paniculata* seems to be an inbreeder plant; it is possible that they act as hermaphroditic and self-compatible plant. Even through the flowers of *A. paniculata* were frequently visited by honeybees, the visitors were often found to carry pollen mass away from the flower. However, the DNA markers investigated in this study revealed a mutual same pattern, providing the support that *A. paniculata* might be first introduced into Thailand in manner of accession before spreading to other regions of the country and acting as inbreeder. Second, it is possible that the observed lack of genetic differentiation in *A. paniculata* may be caused by gene flow rate which is often low in herbaceous plants (Widen et al., 1992) and in such case a distance of more than 30 km is usually sufficient to keep variety isolated in plant breeding (Levin, 1984). The gene flow rate of *A. paniculata* of Thailand may contribute to the observed low of gene dispersion that affects allele frequencies in a random mating population. In a higher plant, gene flow is accomplished by dispersal of seeds and pollens as well as by vegetative mobility (Handel et al., 1985; Parker and Hamrick 1992).

Natural selection therefore decreases the frequencies of alleles that reduce the fitness of an organism and increases the frequency of alleles that improve fitness. In this study, few new alleles found in *A. paniculata* collected from the accessions of PB, NR, BR1 and MK, which unchanged frequencies because of random genetic drift caused by the arbitrary nature of birth, death, and reproduction.

Third, the random genetic drift, which causes local populations to lose genetic diversity but in turn increasing population differentiation as a result of these study that *A. paniculata* act as inbreeder and have to loss of variation (Lande, 1976).

Comparison between marker systems

Although SSR have been previously reported that it is a powerful marker technique because it is multi-allelic. It is also known that when SSR have been compared to other marker systems they have revealed the highest level of polymorphism (Morgante et al., 1994; Powell et al., 1996; Wu and Tanskley, 1993). These features of SSR are particularly attractive for distinguishing between cultivars because the level of polymorphism detected at SSR loci is higher than that detected with any other molecular marker assay (Powell et al., 1996). However, in this study, all of 58 collecting samples of *A. paniculata* were relatively monomorphic for the 70 SSR markers used as shown by the high molecular genetic similarity. Moreover, AFLP and RAPD showed similar results in identical DNA profiles of undifferentiation for all samples. It is interesting to note that the most common genotype of *A. paniculata* in Thailand may have arisen through the establishment of only one individual genotype different from those others of geographically distribution in many parts of Asia. In this study, only alleles of the seventy SSR markers (APEII8-5F, AP7) could detect all of 58 *A. paniculata* collecting samples. The four cultivars collected from PB (Central), NR1, BR1 and MK (Northeast) were shown polymorphic but another 54 collecting samples were monomorphic. Phylogenetic trees analysis shown by genetic similarity index indicates that they are highly similar although the trees from RAPD marker system seemed to have the lowest correlations among other marker systems (Powell et al., 1996). In this respect, it has been shown that RAPDs analysis, based on random primed amplifications, is likely to suffer from a lack of reproductivity due to mismatch annealing (Neale and Harry, 1994).

Based on the results of this study, AFLP markers showed higher resolution than SSR and RAPD. This technique, however, provided only a total of 8(9.41%) polymorphic bands allowing the differentiation of 6 out of 58 collecting samples. We have observed that the most related individuals, belonging to the same varieties in 58 samples excepted NP, NR, BR1, MK, SK2 and SO1 collecting samples, were different from intrapopulation which could be due to the natural mutation or genetic drift process.

The RAPD technique resulted in higher polymorphic than SSR and AFLP. This event could be due to the amount of variability detected with RAPD analysis is dependent upon the selection of appropriate primers.

This technique has the advantage of being inexpensive to perform, and does not require a previous knowledge of the genome. Similar level of genetic similarity estimates were obtained using the SSR, AFLP and RAPD markers in this study indicating that all three molecular marker systems showed a similar discriminatory power and revealed the extremely presence of a single varieties of *A. paniculata* in Thailand. The SSR marker is the most stable amplification while RAPD is the least stable markers, whereas the AFLP is in between the two types.

Genetic diversity of Thai *A. paniculata*

For SSR markers, 58 Thai collecting samples clustered into only 1 group at 97% genetic similarity. The Shannon diversity index calculated for the studied population was 0.067 indicating a low level of genetic diversity of *A. paniculata* in Thailand. As previously reported by Padmesh et al. (1998), *A. paniculata* populations in different countries were reported to differ from the Thai population, suggesting that almost all accessions of *A. paniculata* in India cannot be genetically similar to genotypes in all of the studies, except one accession being similar to the possible Indian provenance of Indian *A. paniculata* since it is an introduced species in Thailand. Furthermore, this study has a similar result in genetic evaluation of Thai *A. paniculata* accessions, as found in previous report by Maison et al. (2005) and Sakuanrungsirikul et al. (2008)

With AFLP markers, the four AFLP primer combinations produced a total of 85 bands. The only 15 polymorphism bands were detected. Our result agrees with similar studies by SSR markers revealing that *A. paniculata* from Thailand indicated a low level of genetic diversity.Based on the results of RAPD markers, all the 58 collecting samples of *A. paniculata* were the most polymorphic markers of those evaluated, and consequently had the most resolving power. Although the amount of variability detected with RAPDs, RAPD markers do not contributed to several clusters. The cluster analysis confirmed that *A. paniculata* clustered into only 1 group at 91% genetic similarity, as shown by the results of SSRs and AFLPs.

Form the results of these marker systems; we observed that they also shared the same among several collecting samples of *A. paniculata* which contributed into different band. All of the three marker systems provided a shared different DNA band patterns in collecting samples that are derived from various regions of Thailand such as BR1 (Buriram), MK (Maha Sarakham) from Northeastern region.

Our results therefore suggested that the genetic structure of *A. paniculata* is mainly influenced by the combined effects of random genetic drift, restricted gene flow and differential selection pressures (Holderegger and Schneller, 1994). These effects lead to low genetic variation in species within population of *A. paniculata* collecting samples from various regions of Thailand, as found in a study by Nevo (1983).

Conclusion

This study has demonstrated that the SSR, AFLP and RAPD markers have similarly proven to be a promising approach for evaluating genetic diversity of Thai *A. paniculata*, which revealed that all of 58 collecting samples from various locations of Thailand are the same varieties. This may due to the genetic drift even as well as the extremely high self-pollinated nature of the species. The measure of *A. paniculata* provides a deep insight into genetic diversity and valuable information for further germplasm conservation and breeding program.

ACKNOWLEDGEMENTS

This research work was supported by the Royal Golden Jubilee PhD Program (Grant PHD/0047/2546), the Thailand Research Fund (TRF). The study was supported in part by DNA Technology Laboratory, Center for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA).

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